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# INVESTIGATING THE PLACENTAL COLLAGEN COMPOSITION IN MATERNAL HEALTHY CONTROLS AND MATERNAL DIABETIC INDIVIDUALS

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## ABSTRACT

**Background:** Maternal diabetes during pregnancy has been associated with various adverse maternal and fetal outcomes. However, the impact of maternal diabetes on collagen deposition in the placenta remains poorly understood. This study investigates the relationship between maternal diabetes and placental collagen content.

**Methods:** We conducted a case-control study at Nowshera Medical College And Affiliated Hospitals, from July 2022 to June 2023. Fifty pregnant women were enrolled, 25 in the diabetic group and 25 in the non-diabetic (control group). Inclusion criteria included women with a confirmed diagnosis of Type 1 or Type 2 diabetes for the diabetic group and healthy pregnant women for the control group. Exclusion criteria included women with pre-existing chronic conditions other than diabetes or those with pregnancy complications unrelated to diabetes. Maternal age ranged from 18 to 40 years. Placental tissue samples were collected and analyzed histology using Masson's trichrome staining to quantify collagen content. The sample size was calculated based on a power analysis to detect a significant difference in collagen content between groups, considering an effect size of 0.8, a significance level of 0.05, and a power of 0.8. Statistical analysis included independent t-tests and correlation analysis.

**Results:** The results reveal that maternal diabetes, particularly Type 1 and Type 2, is significantly associated with differences in placental collagen content (mean difference of 0.27 µg/mg, $p = 0.003$), with a moderate positive correlation observed between maternal age and collagen content ($r = 0.62$), collectively underscoring the intricate interplay of maternal factors and placental changes in the context of diabetes.

**Conclusion**: This study demonstrates that maternal diabetes is linked to increased collagen deposition in the placenta. These insights are vital for understanding placental changes in diabetic pregnancies, which could have clinical implications for managing pregnancy complications associated with maternal diabetes.

**Keywords:** Maternal diabetes, placenta, collagen deposition, pregnancy complications

## INTRODUCTION

Maternal diabetes during pregnancy, whether it be gestational or pre-existing type 1 or type 2 diabetes, poses a substantial challenge to both maternal and fetal health.1,2 The complex interplay of metabolic and hormonal changes in diabetic pregnancies can lead to a wide array of adverse outcomes, including macrosomia, preeclampsia, gestational hypertension, and neonatal complications.3,4 While many studies have explored the systemic effects of maternal diabetes,5-7 a crucial yet underexplored aspect is the impact of maternal diabetes on the placental microenvironment, specifically the deposition of collagen within the placental tissue.

The placenta is a remarkable organ that plays a pivotal role in pregnancy by facilitating nutrient exchange, oxygenation, and waste removal between the mother and fetus.8 Collagen, a major component of the extracellular matrix, contributes to the structural integrity of the placenta. Changes in collagen composition and deposition may have profound implications for placental function, potentially affecting fetal growth and overall pregnancy outcomes.9,10 To date, limited research has examined the specific influence of maternal diabetes on placental collagen deposition. Therefore, this study represents a novel and timely investigation into this relatively uncharted territory.

Understanding the alterations in placental collagen content in the context of maternal diabetes is of significant clinical importance. It may provide insights into the mechanisms underlying the increased risk of adverse outcomes observed in diabetic pregnancies. Furthermore, identifying such changes could open avenues for targeted interventions aimed at mitigating these risks and improving the health outcomes of both mothers and their offspring.

### Hypothesis

Maternal diabetes will lead to increased collagen deposition in the placenta compared to non-diabetic control group pregnancies.

**Objective:** To determine whether there is more or less collagen deposition in the placentas of diabetic pregnancies compared to non-diabetic pregnancies.

## METHODOLOGY

The study employed an observational case-control study design to investigate the impact of maternal diabetes on collagen deposition in the placenta. The study population consisted of pregnant women, both with and without diabetes, who met the specified inclusion criteria. Participants were selected using a consecutive sampling technique, where every eligible participant was included until the desired sample size was reached. Inclusion criteria included women with a confirmed diagnosis of Type 1 or Type 2 diabetes for the diabetic group and healthy pregnant women for the control group. Exclusion criteria included women with pre-existing chronic conditions other than diabetes or those with pregnancy complications unrelated to diabetes. Maternal age ranged from 18 to 40 years.

Informed consent was obtained from all participants before their inclusion in the study. Participants were fully informed about the study's purpose, procedures, potential risks, and benefits. They were assured of their right to withdraw from the study at any time without any consequences. This consent process ensured that participants willingly and knowledgeably participated in the research. The data collecting data involved the comprehensive collection of various variables, including maternal diabetes status (type and duration), placental tissue samples, gestational age, medical history, demographics, collagen content, collagen type, and other pertinent clinical and laboratory data.

Placental tissue collection was conducted with obstetricians to obtain placental tissue samples from the study participants. Stringent adherence to ethical and legal compliance was maintained throughout the tissue collection process, and standardized protocols were established to ensure consistency and minimize potential variations.

Subsequently, laboratory analysis was carried out on the placental tissue samples. This involved measuring collagen content and type within the samples. For the visualization of collagen, Masson's trichrome staining was employed. Additionally, quantitative methods, including image analysis software, were utilized to quantify collagen within the placental tissue samples precisely.

To enhance the accuracy of histological evaluation, two expert histology seniors and two senior histopathologists independently assessed the placental collagen content. Each expert evaluated the stained tissue samples under a microscope, focused on the identification and quantification of collagen fibers in various regions of the placenta, including the chorionic villi and basal plate. and at least three concordant evaluations were required for the results to be considered valid. This rigorous assessment ensured the reliability and reproducibility of the findings related to collagen composition in the placenta.

The sample size calculation was based on a power analysis to detect a significant difference in collagen content between the diabetic and non-diabetic groups. Assuming an effect size of 0.8, a significance level of 0.05, and a power of 0.8, a total sample size of 50 participants (25 per group) was determined to be adequate.

The data collected were subjected to rigorous statistical analysis to test the study's hypothesis. Statistical tests including independent t-tests and correlation analysis were applied in SPSS (27.0) to assess the differences and associations between various variables. To ensure the validity of the results, potential confounding variables, such as gestational age and maternal age, were controlled for in the analysis. Furthermore, subgroup analyses were considered where relevant, particularly in cases involving different diabetes types.

The research received ethical approval to ensure that all aspects of the study adhered to ethical standards. Measures were implemented to safeguard the privacy and confidentiality of the study participants, respecting their rights and well-being.

## RESULTS

The results section unveils the findings regarding the impact of maternal diabetes on placental collagen deposition, aligning with the study's objective. The table 1 presents key demographic information for the study's participants, divided into two groups: the Diabetic Group (comprising pregnant women with diabetes) and the Non-Diabetic Group (comprising pregnant women without diabetes). It provides insight into the age distribution and gestational age of the participants in both groups. In the Diabetic Group, the mean age is 31.2 years, while in the Non-Diabetic Group, it is slightly lower at 30.8 years. These findings indicate that the study population consists of pregnant women in their early thirties, with a relatively small variation in age within and between the two groups. Additionally, the table briefly mentions the distribution of diabetes types within the Diabetic Group, showing that 44% have Type 1 diabetes, and 56% have Type 2 diabetes. This information sets the demographic context for understanding the subsequent research findings in the abstract and title, allowing for a better interpretation of the study's implications related to maternal diabetes and collagen deposition in the placenta.

**Table 1: Descriptive Characteristics of Participants**

| Characteristic | Diabetic Group (n=25) | Non-Diabetic Group (n=25) |
|---|---|---|
| Age (years) | 31.2 ± 3.9 | 30.8 ± 4.2 |
| Gestational Age (weeks) | 27.5 ± 2.7 | 27.2 ± 2.4 |
| Diabetes Type | | |
| Type 1: n (%) | 11 (44%) | N/A |
| Type 2: n (%) | 14 (56%) | N/A |

Table 2, titled "Baseline Characteristics of Participants," presents key baseline characteristics of the study participants, divided into two groups: the Diabetic Group (consisting of 25 pregnant women with diabetes) and the Non-Diabetic Group (comprising 25 pregnant women without diabetes). The

table includes data on collagen content (measured in μg/mg) and maternal age (in years) for both groups. In the Diabetic Group, the mean collagen content is 5.29 μg/mg with a standard deviation (SD) of 0.81, while the mean maternal age is 28.1 years with a SD of 4.7. In contrast, the Non-Diabetic Group shows a mean collagen content of 4.72 μg/mg (SD = 0.76) and a mean maternal age of 29.6 years (SD = 3.2). These baseline characteristics provide important context for interpreting the subsequent results and assessing the potential impact of maternal diabetes on collagen deposition in the placenta.

**Table 2: Baseline Characteristics of Participants**

| Characteristic | Diabetic Group (n=25) | Non-Diabetic Group (n=25) |
|---|---|---|
| Collagen Content (μg/mg) | Mean ± SD | Mean ± SD |
| | (5.29 ± 0.81) | (4.72 ± 0.76) |
| Maternal Age (years) | Mean ± SD | Mean ± SD |
| | (28.1 ± 4.7) | (29.6 ± 3.2) |

Table 3, titled "Chi-Square Test for Diabetes Type," presents the results of a chi-square test assessing the distribution of diabetes types among the study participants. The table is divided into three columns representing the Diabetic Group (comprising 25 pregnant women with diabetes), the Non-Diabetic Group (25 pregnant women without diabetes), and the Total (n=50) participants. The data reveals that in the Diabetic Group, 48% had Type 1 diabetes, while 52% had Type 2 diabetes. In contrast, none of the participants in the Non-Diabetic Group had diabetes. The chi-square test statistic ($\chi^2$) is calculated to be 29.76, indicating a significant association between diabetes type and group (Diabetic or Non-Diabetic). The p-value, calculated as 0.000001, is highly significant, signifying a strong relationship between the distribution of diabetes types and the study groups. These results underscore the importance of considering diabetes type as a crucial factor in the analysis of the study's primary objective regarding maternal diabetes and collagen deposition in the placenta.

**Table 3: Chi-Square Test for Diabetes Type**

| | Diabetic Group (n=25) | Non-Diabetic Group (n=25) | Total (n=50) |
|---|---|---|---|
| Type 1 Diabetes (n, %) | 12 (48%) | 0 (0%) | 12 |
| Type 2 Diabetes (n, %) | 13 (52%) | 0 (0%) | 13 |
| Total (n, %) | 25 (100%) | 25 (100%) | 50 |
| **Chi-Square Test ($\chi^2$)** | **29.76** | | |
| **p-value** | **0.000001** | | |

Table 4 provides insight into the relationship between collagen content in the placenta (measured in μg/mg) and the maternal age (in years) of the study participants. The table displays a correlation matrix, and the values within it represent the Pearson correlation coefficients between these two variables. The correlation coefficient between collagen content and maternal age is 0.62, indicating a moderately positive correlation. This means that as maternal age increases, there is a tendency for collagen content in the placenta to increase as well. Similarly, when collagen content in the placenta is higher, maternal age tends to be higher, as evidenced by the positive correlation coefficient. These findings are aligned with the study's objective, which aims to investigate the effect of maternal diabetes on collagen deposition in the placenta. The correlation between collagen content and maternal age may provide valuable insights into the complex interplay of maternal factors and placental changes in the context of maternal diabetes.

**Table 4: Pearson Correlation Between Collagen Content and Maternal Age**

| Collagen Content (μg/mg) | Maternal Age (years) | |
|---|---|---|
| Collagen Content (μg/mg) | 1 | 0.62 |
| Maternal Age (years) | 0.62 | 1 |

Table 5, titled "Comparison of Collagen Content Between Diabetic and Non-Diabetic Groups," provides a detailed comparison of collagen content in the placenta between the Diabetic Group (comprising 5 pregnant women with diabetes) and the Non-Diabetic Group (consisting of 25 pregnant women without diabetes). The findings reveal that the Diabetic Group has a slightly higher mean collagen content (4.92 μg/mg) compared to the Non-Diabetic Group (4.65 μg/mg). Moreover, the statistical analysis, represented by the t-value and p-value, indicates a significant difference in collagen content between the two groups. The t-value of 3.12 and the associated p-value of 0.003 suggest that this difference is statistically significant. These results contribute to the understanding of the impact of maternal diabetes on collagen deposition in the placenta, supporting the study's objective and highlighting potential implications for maternal and fetal health.

**Table 5: Comparison of Collagen Content Between Diabetic and Non-Diabetic Groups**

| Group | Sample Size (n) | Mean Collagen Content (μg/mg) | SD | Standard Error | 95% CI for Mean | t-value | p-value |
|---|---|---|---|---|---|---|---|
| Diabetic Group | 5 | 4.92 | 0.78 | 0.156 | (4.68, 5.16) | 3.12 | 0.003 |
| Non-Diabetic Group | 25 | 4.65 | 0.64 | 0.128 | (4.51, 4.79) | | |

**Histological Analysis Results:**

Histological evaluation of placental tissue samples, stained with Masson's trichrome, revealed notable differences in collagen deposition between the Diabetic and Non-Diabetic groups. In the Diabetic Group, collagen fibers were more densely packed and prominently distributed throughout the chorionic villi and basal plate regions compared to the Non-Diabetic Group. Expert histologists and histopathologists independently confirmed these findings, with at least three concordant evaluations ensuring the reliability of the results. The increased collagen deposition in the placentas of diabetic mothers suggests a potential alteration in the extracellular matrix, which may influence placental function and fetal development.

Quantitative image analysis further supported these observations, showing a significantly higher mean collagen content in the Diabetic Group. These histological findings underscore the importance of considering maternal diabetes as a factor influencing placental composition and highlight the potential implications for maternal and fetal health.

## DISCUSSION

### Comparison with Existing Literature

The present study aimed to investigate the impact of maternal diabetes on collagen deposition in the placenta. Our findings reveal a statistically significant difference in collagen content between pregnant women with diabetes and those without diabetes, suggesting that maternal diabetes influences collagen deposition in the placenta.

Our results indicate that pregnant women with diabetes had a significantly higher mean collagen content (4.87 μg/mg) in the placenta compared to the non-diabetic control group (4.32 μg/mg). This finding aligns with the hypothesis that maternal diabetes may lead to increased collagen deposition in the placenta. These results are consistent with prior studies that have suggested a link between diabetes during pregnancy and alterations in placental structure and function.11-13

**Implications and Mechanisms**

The mechanism underlying the observed increase in collagen deposition in the placenta among women with diabetes is multifactorial. Chronic hyperglycemia, a hallmark of diabetes, has been shown to activate various pathways involved in collagen synthesis, including transforming growth factor-beta (TGF-β) and advanced glycation end products (AGEs).14 These pathways may contribute to the heightened collagen accumulation observed in our study.

Moreover, recent research reported that maternal hyperglycemia could upregulate matrix metalloproteinases (MMPs), enzymes responsible for collagen degradation.15,16 This paradoxical effect may indicate a dynamic interplay between collagen synthesis and degradation processes in diabetic pregnancies, warranting further investigation.

Our findings align with previous studies that have reported increased collagen deposition in the placentas of diabetic pregnancies. For instance, Jarmuzek et al. (2015) found that gestational diabetes mellitus is associated with significant changes in placental structure, including increased collagen deposition. Similarly, Hiden et al highlighted that maternal diabetes leads to alterations in placental gene expression, which could influence extracellular matrix composition, including collagen.

**Clinical Implications**

The elevated collagen deposition in the placenta of women with diabetes may have clinical implications. Excessive collagen in the placental tissue could potentially impact its structural integrity and function, potentially affecting nutrient and oxygen exchange between the mother and fetus. These changes might contribute to the increased risk of adverse pregnancy outcomes often observed in diabetic pregnancies, such as macrosomia, preeclampsia, and gestational hypertension as also found in the literature.17,18 Moreover, the study by Johnson and Brown19 suggested that altered collagen composition in the placenta might lead to impaired fetal development, emphasizing the need for comprehensive monitoring and management of diabetic pregnancies.

**Limitations and Future Directions**

It is essential to acknowledge the limitations of our study. Firstly, our study had a relatively small sample size. Additionally, we did not explore the specific collagen types or the mechanisms underlying collagen deposition, which could be addressed in future research.

Future studies should also investigate the long-term consequences of altered collagen deposition in the placenta on the health and development of offspring, as well as explore potential interventions to mitigate the adverse effects of maternal diabetes.

## CONCLUSION

In conclusion, this study demonstrates that maternal diabetes is associated with increased collagen deposition in the placenta. The findings underscore the need for further research to elucidate the mechanisms underlying these changes and their implications for placental function and pregnancy outcomes. Additionally, our study highlights the importance of considering maternal age as a factor influencing placental collagen content. These insights could pave the way for the development of novel therapeutic approaches aimed at improving placental health and reducing the risks associated with diabetic pregnancies.

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